Bacteria Source Tracking in Johnson Creek and Major Tributaries

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Introduction

The purpose of this study was to determine the concentrations of summer bacteria pollution throughout the Johnson Creek watershed and to identify whether human fecal contamination was present. The data would allow the Johnson Creek Inter-jurisdictional Committee[1] (IJC) and the Johnson Creek Watershed Council to focus future actions to reduce bacteria pollution, with the goal of meeting the water quality criterion for primary contact recreation (<406 *Escherichia coli* (*E. coli)* organisms per 100 ml for grab samples and less than an average of 126 *E. coli* organisms per 100 ml over time). This Oregon State water quality criterion was based on the risk of gastrointestinal illness for people swimming in freshwater water bodies. This study was funded with $16,000 of a $45,000 319 Non-point Source grant from the Oregon Department of Environmental Quality in 2013 (Grant # X).

Background

Johnson Creek flows 26 miles from its headwaters near Boring, Oregon to its confluence with the Willamette River, passing through rural agricultural areas, five cities (Gresham, Portland, Milwaukie, Damascus, and Happy Valley), and two counties (Clackamas and Multnomah) along the way (see map, Figure 1). Numerous springs and between 36 and 50 inches of annual rainfall over the 54 square-mile watershed provide stream-flow to the creek and its tributaries. Significant tributaries to Johnson Creek include Crystal Springs, Kelley, Butler, Hogan, Sunshine, and Badger Creeks. Salmon, trout, diverse wildlife, and over 180,000 people live in the Johnson Creek watershed. In addition to the over 2,000 acres of parks and natural areas in the watershed, the public can access the creek from the 21-mile long Springwater Corridor trail and road crossings at numerous locations.

Johnson Creek has consistently exceeded the Oregon water quality criteria for *E. coli* bacteria, similarly to many urban streams in the Portland area, including Fanno and Tryon Creeks. DEQ's general water quality criteria require that no single sample can exceed 406 *Escherichia coli* (*E. coli)* organisms per 100 ml, and for a minimum of five samples the 30-day log mean cannot exceed 126 *E. coli* organisms per 100 ml. Due to high bacteria levels, Johnson Creek was included in the 2006 Lower Willamette Subbasin TMDL for bacteria (ODEQ, 2006). The TMDL load and waste load allocations for bacteria require a 78% reduction to meet the numeric criteria of 126 cfu/100 ml. While the point-source bacteria reductions are addressed via National Pollutant Discharge Elimination System (NPDES) permits and Agricultural Water Quality Management Area Plans address the non-point sources of bacteria, insufficient data exist to identify sources spatially and prioritize this work.

[1] The IJC is made up of technical staff from the JCWC and jurisdictions and agencies that manage portions of the watershed, including the U.S. Geological Survey, Oregon Department of Environmental Quality, Oregon Department of Agriculture, the Cities of Damascus, Gresham, Milwaukie and Portland, Multnomah and Clackamas Counties, the East Multnomah and Clackamas Soil and Water Conservation Districts, and volunteer retired scientists and active Portland State University graduate students.

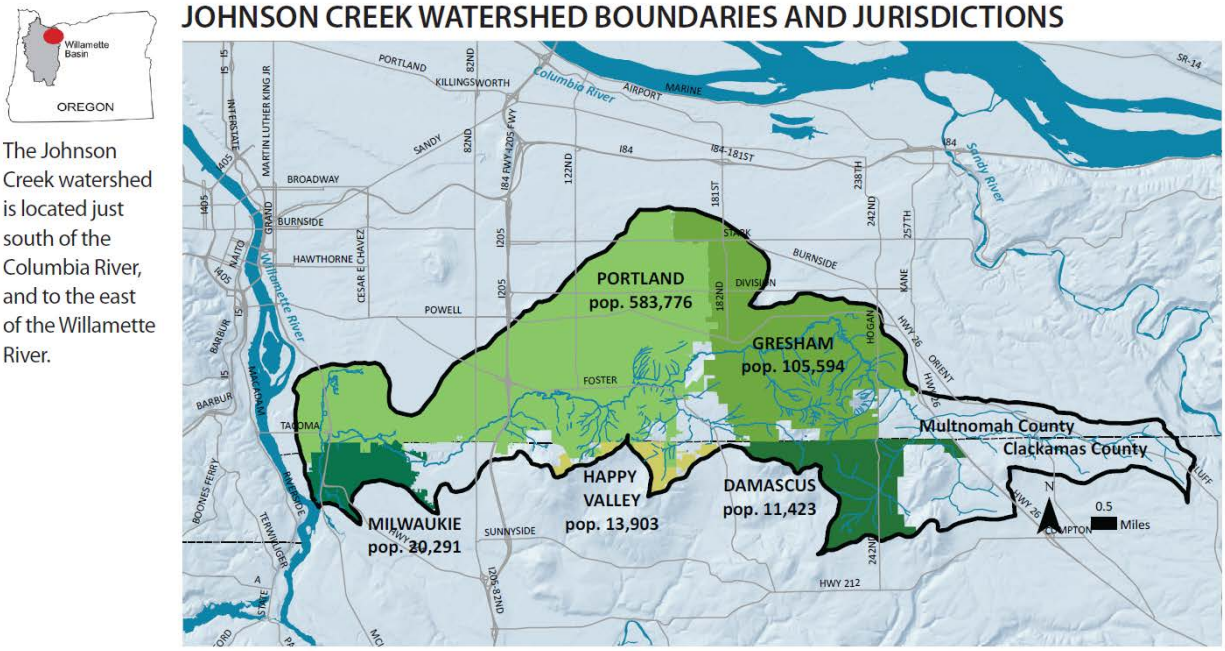

Figure 1: Location of the Johnson Creek watershed.

Ambient monitoring by local jurisdictions has shown small decreases in bacteria concentrations in the mainstem of Johnson Creek since the TMDL plans were implemented. In Figure 2, the magenta and blue bars show *E. coli* levels from two, seven-year periods between 1996 and 2010. It is apparent that the geometric means of bacteria concentrations are mostly lower in the more recent time period, although levels still show great seasonal variations.

Specifically, *E. coli* is higher in summer months, a critical time when people are most likely to come in contact with water in Johnson Creek and risk exposure to fecal pathogens. Because *E. coli* levels in Johnson Creek remain elevated over a range of streamflows (Figure 3), a number of different sources of fecal contamination are probable. General sources of *E.coli* in surface waters are well-documented in studies from the Pacific Northwest and across the nation (Shanks et al., 2006; Clean Water Services, 2005; Tetra Tech and Herrera, 2011). Birds, rodents, pets, livestock, and human sources are among the most prevalent sources in urban and rural residential areas. Winter runoff can carry manure from livestock into streams as well as bacteria from wildlife sources and pets. Dry weather contamination may result from septic system failures or sewer cross connections. Of all *E. coli* sources, the human source is potentially the most important to control. The presence of human-derived bacteria, as opposed to most other animal sources, carries the highest disease risk from accompanying pathogens to people coming in contact with water (Soller et al., 2010).

While the long-term dataset from several fixed sites in the watershed has been useful in determining *E. coli* bacteria concentration status and trends, *E. coli* is not monitored at a fine enough scale to detect tributary contributions. Second, it does not provide information on specific sources (e.g., bird, livestock, human) of fecal contamination. And third, for a number of reasons, the use of *E. coli* as a primary indicator of fecal contamination in waterways is now under question (Sauer et al., 2011), and Johnson Creek jurisdictions were curious about alternative indicators.

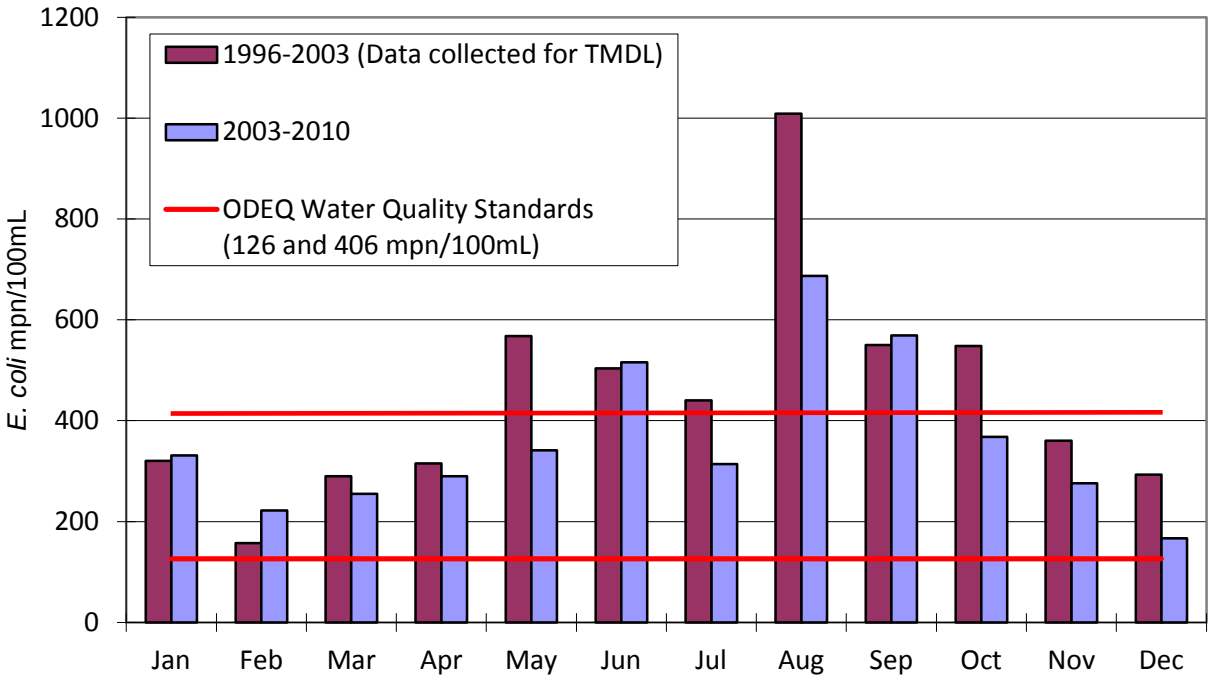

Figure 2. Distribution of monthly *E. coli* geometric mean concentrations collected between 1996-2003 and 2003-2010.

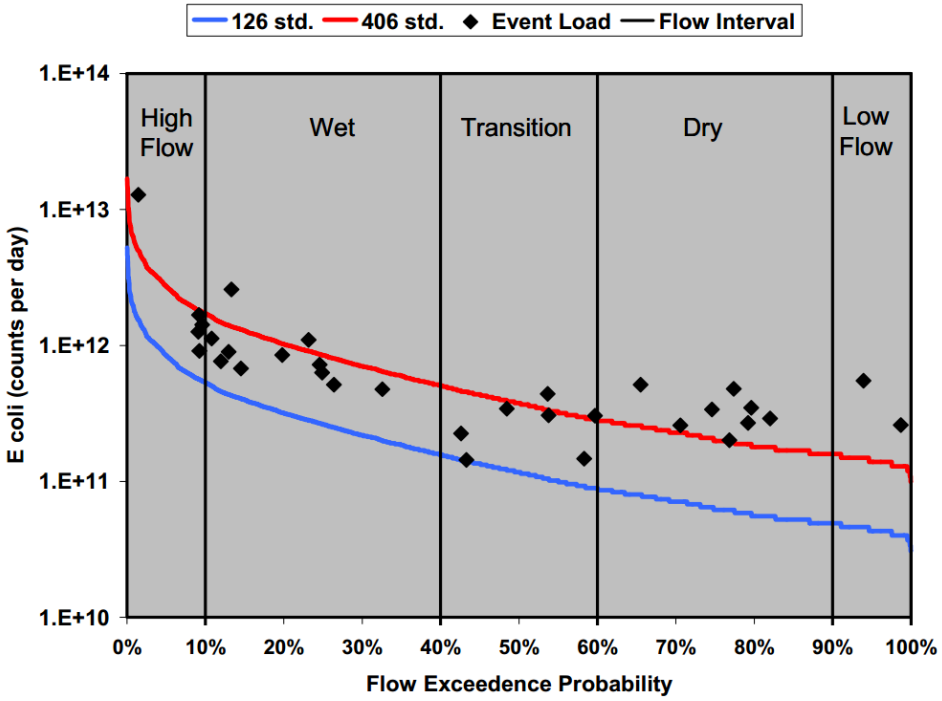

Figure 3. *E. coli* load duration curve for Johnson Creek at 17th Ave (from ODEQ 2006, page 5-119).

Methods

Finer-Scale Monitoring and Tributary Contributions of E. coli

Our sampling was intended to characterize summer bacteria concentrations, relative bacteria loads from various stream reaches and identify sources in Johnson Creek when public risk of exposure to pathogens is greatest. The lack of finer-scale and tributary bacteria data was the impetus for a basin-wide bacteria blitz to collect synchronous samples and measure *E. coli* levels from a greater density of sites throughout the Johnson Creek watershed in late summers, 2012 and 2013. Simultaneously, the U.S. Geological Survey collected streamflow measurements in the mainstem and sampled at the mouths of perennial tributaries to estimate bacteria loading.

This project involved collecting water samples at 70 sites distributed throughout the Johnson Creek watershed. The sites were selected to represent different land uses and to determine the contribution of perennial tributaries. Sites were selected at road crossings and public land for accessibility. There were 29 mainstem and 41 tributary sites sampled in the morning of August 13, 2012 (Figure 4). On August 27, 2013, a second morning bacteria blitz was conducted following several weeks of dry weather at 70 sites to determine whether similar summertime *E. coli* levels were present as in 2012. Of these 70, 28 were mainstem sites and 42 were tributaries. The only differences between the 2012 and 2013 sites were the addition of tributary sites at Wahoo Creek and Butler Creek at Towle/Butler Rds, and the removal of a redundant site on Johnson Creek at the mouth of Deardorff Creek.

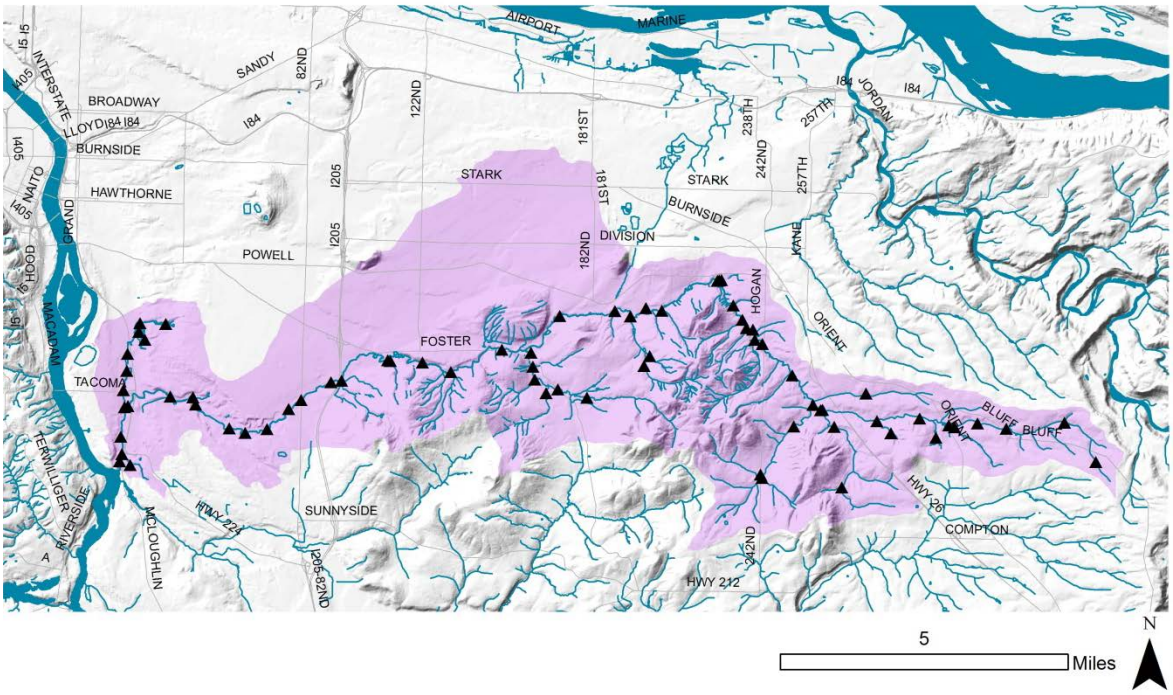

Figure 4. Johnson Creek watershed map showing sampling locations for 2012 bacteria study.

Samples were collected to determine the *E. coli* concentration and for source identification. Two grab samples were collected at all sites shown in Figure 4 on the morning of August 13, 2012, which was preceded by three weeks of dry weather. Samples were collected in a well-mixed area near the center of the stream channel. Samples were collected directly from the stream into sterile sample bottles labeled with the sample site, date, time, and sample collector(s), and transported on ice to a mobile

laboratory. Precision of grab samples for *E. coli* detection was evaluated by collecting an additional duplicate sample at 10% of the sites.

One grab sample from each site plus the duplicates were processed using IDEXX Quanti-Trays and Colilert reagent to determine the most probable number of *E. coli* using the Oregon DEQ mobile laboratory, which was parked at a central location in the watershed to minimize handling time: Gresham's Main City Park. Incubator temperatures were checked at the beginning and end of the incubation and recorded in a log book kept with the incubator along with date, time and name of person who completed the check (Larry Marxer, Oregon DEQ). Results of the sampling were available within 24 hours.

The second grab sample from each site was packaged in paper towels and Ziploc bags and mailed on ice in coolers overnight to the Source Molecular Corporation laboratory for host identification using the quantitative Polymerase Chain Reaction (qPCR) methodology.

Microbial Source Tracking

After reviewing a number of methods and other studies (Tetra Tech and Herrera, 2011; Fields study), we decided to use the cost-effective PCR and qPCR methods to identify human and avian fecal contributions to Johnson Creek and tributaries. Recent advances in Microbial Source Tracking (MST) have led to a greater reliance on this type of genetic marker associated with particular animal feces, rather than previous costly and geographically-limited library-dependent MST methods (Boehm et al., 2013). "qPCR is a molecular biology tool that amplifies the DNA of specific genes and allows for quantification of as low as one gene copy. Additionally, qPCR offers the ability to identify specific microbial hosts through the quantification of gene sequences in fecal indicator bacteria strains unique to target species" (Trapp, Burge, and Libes, 2012). PCR and qPCR tools can also be used in tandem with the newly approved U.S. EPA bacteria monitoring methods (EPA, 2010).

Bacteroides as an Alternative Indicator of Fecal Contamination

Although E. coli is the Oregon State-mandated indicator of waterborne health risk due to fecal contamination, it has been shown to be inadequate in several regards (Walters and Field, 2006).

- *E. coli* may naturalize in certain conditions, allowing it to persist and in some cases multiply in an aquatic environment. Therefore, when used as an indicator, it can signal the presence of pathogens long after the health hazard has subsided or can signal higher levels of pathogens than are actually present.
- Culturing techniques required for quantification of *E. coli* take a minimum of 24 hours, causing recreational warnings to be posted after danger may have passed.
- *E. coli* tests provide no information on potential host sources.

As an alternative, Bacteroides, a genus within the phylum Bacteroidetes, is one of the most common and abundant bacteria found in the guts of warm-blooded organisms. Unlike E. coli and other coliforms, Bacteroides is anaerobic and so is not capable of multiplication in most natural aquatic environments. In contrast to E. coli, "reservoirs for Bacteroides bacteria are believed to be restricted to the body cavities of animals; no strains adapted to aquatic environments are currently known" (Walters and Field, 2006). Moreover, specific species of Bacteroides have shown a high degree of host specificity (Walters and Field, 2006). Furthermore, unlike *E. coli*, Bacteroides DNA is very short-lived in water bodies, with a half-life of 1-3 days (decreasing with increasing temperature). "Thus, the presence of Bacteroides DNA in a water sample is thought to be more indicative of a recent and direct fecal incursion into the water than the presence of *E. coli*" (Pikaart, 2012).

Tests for Bacteroides in surface water rely not on culture of live organisms but on molecular detection of the bacteria's genomic DNA. This testing is done by isolation of DNA from an environmental sample and testing that DNA by quantitative PCR (qPCR). Because qPCR relies on detection of particular DNA sequence, selection of appropriate target sequences can distinguish the subtle differences in DNA not only between one bacterial species and another but, in the case of Bacteroides, between different sub-strains based on the host mammal species (Pikaart, 2012).

Importantly, genetic tests for Bacteroides and Bacteriodales (the family of bacteria of which Bacteroides is a genus) have recently been approved by the U.S. EPA as an alternative to *E. coli* testing under the Clean Water Act, although the type of General Bacteroides test used in this study was slightly different. Method B describes a quantitative polymerase chain reaction (qPCR) procedure for the detection of DNA from Bacteroidales bacteria in ambient water matrices based on the amplification and detection of a specific region of the 16S ribosomal RNA gene from these organisms. Results can be obtained by this method in 3-4 hours, allowing same-day notification of recreational water quality. Recent epidemiological studies at fresh water recreational beaches have demonstrated similar or improved positive correlations between Bacteroidales DNA measurements by this method and swimming associated gastrointestinal (GI) illness rates (U.S. EPA, 2010).

Source Molecular Corp. analyzed 50 of the mailed samples for total General Bacteroides ID. Forty-eight samples with high General Bacteroides levels were identified as candidate samples for source identification. These samples were tested for one of the three Bacteroides species commonly associated with humans (Human Bacteroides-based Marker analysis, *Bacteroides dorei*), which has a 92 to 94% allegiance to human guts (http://www.ncbi.nlm.nih.gov/pubmed/22809116; and http://www.sciencedirect.com/science/article/pii/S0043135413005502). Those that tested positive for this indicator were further tested for two additional Human Indicators (*B. stercoris*, and *B. thetaiotaomicron)*. Avian Fecal ID analysis was also conducted for these 48 samples.

Results and Discussion

E.coli concentrations

There was a wide range of *E. coli* concentrations and *Bacteroides* spp. found in the August, 2012 samples. *E. coli* concentrations ranged from 10 organisms/100ml to more than 2420 organisms/100ml (upper limit of IDEXX *E.coli* method without dilution). Figure 5 shows a comparison of 2012 and 2013 *E. coli* data. It was not unexpected that the results from 2012 and 2013, while in the same range, do not match up very well in absolute concentrations but match up reasonably well in relative concentrations. All sites on the mainstem that had bacteria concentrations >406 MPN/100 mL in 2012 had bacteria concentrations >406 MPN/100 mL in 2013. In the tributaries, only eight of 13 sites with high bacteria concentrations in 2012 had high bacteria concentrations in 2013. Overall, the bacteria concentrations in 2013 were slightly higher than in 2012 but compared to the 15+ year record, bacteria concentrations in both years were lower than previously found.

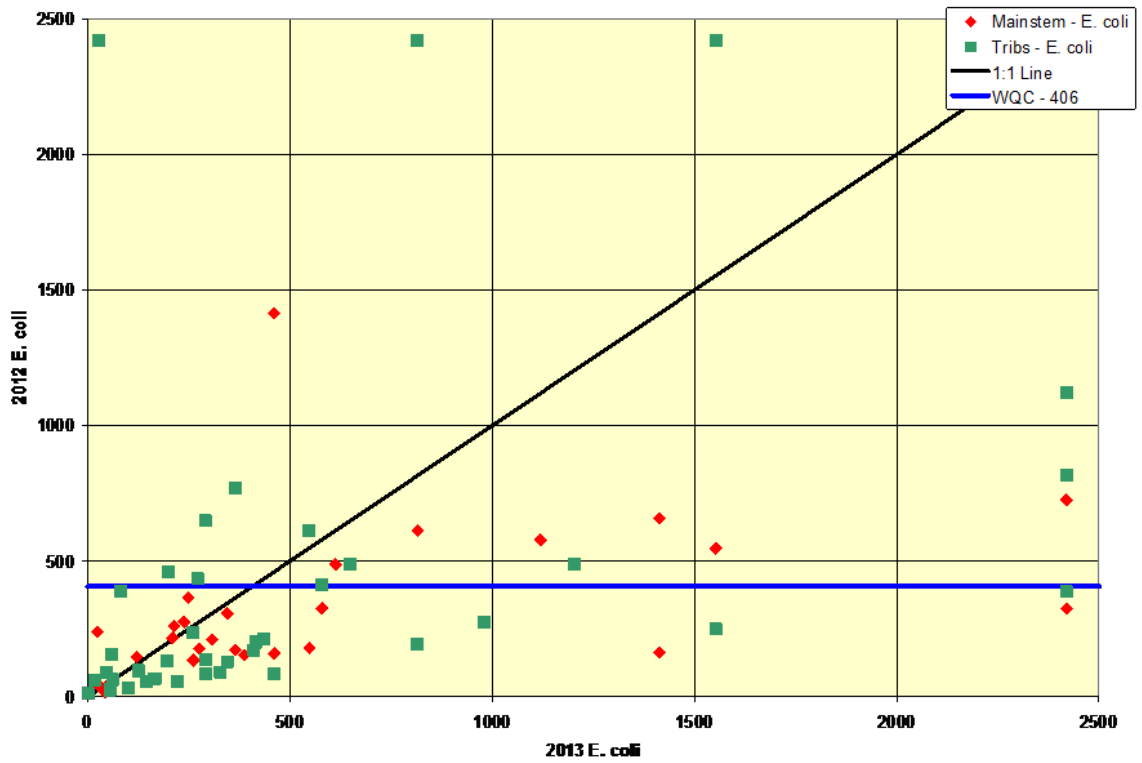

Figure 5. 2012 and 2013 E. coli mpn/100mL results.

The 2012 *E. coli* results were grouped into three categories based on water quality criteria: Green (<monthly geometric mean standard of 126 MPN/100ml); Yellow (>126 but ≤406 MPN/100ml; note that the geometric mean criterion was applied to a single sample); and Red (>single grab criterion of 406 MPN/100ml). Table 1 shows that about one-third of samples exceeded the single grab sample standard (406 MPN/100 mL) for *E. coli* in the mainstem. Tributary *E. coli* concentrations were split evenly between the three color categories.

Bacteroides concentrations

Bacteroides concentration in the mainstem were split into arbitrary groupings based on the general distribution of values, with the Yellow group containing the greatest number of sites (Table 1). For tributary sites, the grouping was dissimilar from that of the *E.coli* concentration, with most sites falling into the Red category. In comparison to mainstem sites, the tributaries had lower *E. coli*, but higher Bacteroides levels.

Table 1: Summary of *E.coli* and General Bacteroides results collected in Johnson Creek mainstem and tributaries in 2012.

	2012		2013	
	Mainstem	**Tributaries**	**Mainstem**	**Tributaries**
E. coli (MPN/100 mL)	n=29	n=41	n=28	n=42
>406	28%	32%	46%	38%
126-406	62%	34%	36%	36%
<126	10%	34%	18%	26%
Bacteroides (cells/100 mL)	n=23	n=27		
>50,000	35%	52%	NM	NM
10,000-50,000	48%	44%	NM	NM
<10,000	17%	4%	NM	NM

E. coli and general *Bacteroides* results are shown by stream mile in Figure 6. From upstream on the left to downstream on the right, the blue and black lines represent *E. coli* and general Bacteroides levels at mainstem sites, respectively, and the dots show bacteria levels at tributary sites along the way. We found no statistically significant correlation between *E. coli* and Bacteroides in Johnson Creek, which is consistent with other studies (e.g. Johnson et al., 2009; Peed et al., 2011). *E. coli* and general Bacteroides concentrations are both relatively low in the headwaters of Johnson Creek, but increase downstream and maintain higher levels for much of its length. While a range of low and high *E.coli* concentrations were found in tributary samples, there were no observed changes in concentration in the mainstem as a result of mixing.

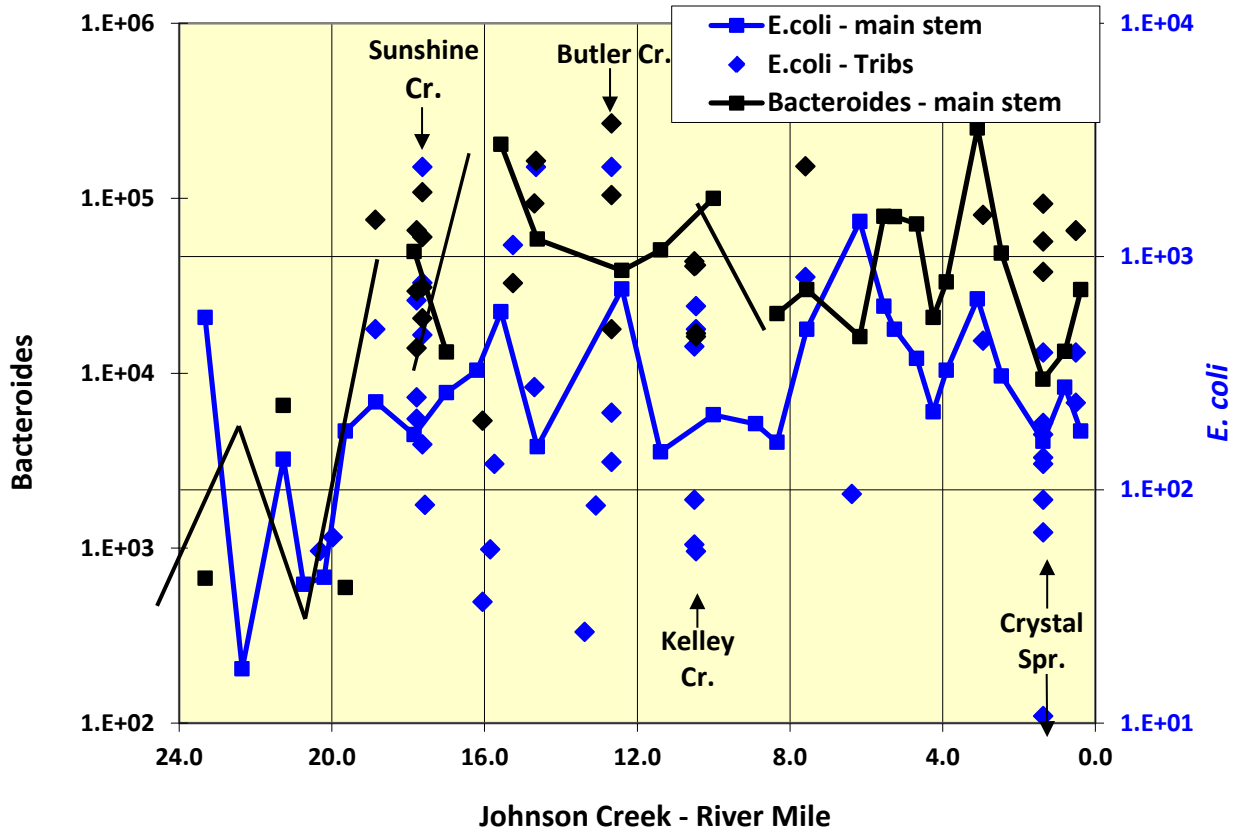

Figure 6. *E. coli* (blue dots/line) and general Bacteroides (black dots/line) concentrations from upstream (left) to downstream (right) in the Johnson Creek watershed.

Load Calculations

At the same time bacteria samples were being collected in 2012 and 2013, the U.S. Geological Survey took tributary streamflow measurements so that bacteria loading could be calculated. Results are shown in Tables 2 and 3 below. The full dataset is attached as Appendix B.

Table 2. Streamflows and Bacteria Loads in Mainstem Johnson Creek in 2012 and 2013.

Site	Location (upstream to downstream)	E. coli (mpn/100ml)		Measured Streamflow (cfs)		E. coli Load (mpn/sec)		Bacteroides (cells/ 100mL)	Bacteroides Load (cells/sec)
		2012	2013	2012	2013	2012	2013	2012	2012
JC07	SE 282nd Ave	238	24	1.1	0.9	76,219	6,320	N/A	N/A
JC11	SE Regner Rd	579	1120	2.0	1.6	324,854	**507,438**	203,000	**113,816,626**
JC15	USGS Sycamore Gage	210	308	3.3	2.6	197,831	226,761	99,900	94,200,715
JC27	JCWC Office/Milport Rd	276	238	18.7	18.0	**1,458,840**	**1,213,094**	13,300	70,426,763

For mainstem Johnson Creek, the E. coli levels were low upstream of SE 282nd Ave, but higher at SE Regner Rd. Between these sites, Badger, Sunshine, and North Fork Johnson Creeks enter the mainstem and may be contributing to the higher bacteria levels at SE Regner Rd. It is interesting to note that *E. coli* levels at the USGS Sycamore gage are not higher, since there is significant beaver activity just upstream. While *E. coli* levels are not very different between the USGS Sycamore gage and the JCWC Office/Milport Rd. gage, the much larger streamflow at the most downstream site means that the bacteria load is much

higher. However, Bacteroides were very low at the Milport Rd. site in 2012, which led to nearly half the Bacteroides load at Milport Rd despite a nine-fold streamflow difference when compared to the SE Regner Rd. site.

Table 3. Streamflows and Bacteria Loads for Tributaries to Johnson Creek in 2012 and 2013. The bold values are relatively high and the asterisks indicate streamflow values derived from the USGS StreamStats tool rather than direct flow measurements.

Location Description (upstream to downstream)	E. coli (mpn/100ml)		Streamflow (cfs)		E. coli Load (mpn/sec)		Bacteroides (cells/ 100mL)	Bacteroides Load (cells/sec)
	2012	2013	2012	2013	2012	2013	2012	2012
Wheeler Ck @ Wheeler Rd	63	61	0.11*	0.11*	1,972	1,900		
Tributary @ SE 287th Ave	488	1203	0.01	0.01*	1,383	3,407	75,100	212,659
Badger Ck @ Springwater Trail	201	416	0.31	0.26	**17,679**	**30,628**	65,600	**5,758,508**
Sunshine Ck @ mouth	461	199	0.39	0.27	**50,922**	**15,215**	30,900	**3,412,460**
North Fork JC @ SE 282nd Ave	86	291	0.21*	0.16	5,012	13,184		
Hogan Ck @ mouth	33	101	0.21*	0.21*	1,939	6,006		
Cedar Ck @ mouth	56	144	0.03*	0.03*	421	1,223		
Thom Ck @ Springwater Trail	1120	>2420	0.05*	0.05*	**17,123**	**34,263**	32,700	499,988
Thompson Ck @ mouth	276	980	0.07*	0.07*	5,543	**19,425**	93,200	1,875,310
Chastain Ck @ mouth	25	55	0.09*	0.09*	639	1,402		
Heiney Ck @ mouth	86	461	0.05*	0.05*	1,193	6,527		
Butler Ck @ SW 14th Ave	132	196	0.13	0.07	4,848	3,774	17,800	655,251
Clatsop Ck @ mouth	411	579	0.003*	0.003	349	492	43,600	37,038
Kelley Ck @ SE 159th Ave	488	649	0.33	0.35	**45,639**	**64,322**	16,200	1,513,817
Jenne Ck @ mouth	58	17	0.12*	0.02	1,913	77	40,900	1,342,244
Frog Ck @ mouth	816	>2420	0.003	0.03	694	**19,187**	152,000	129,125
Veterans @ mouth	96	125	0.22	0.01	5,974	389		
Errol Springs Ck @ mouth	435	272	0.81	0.84	**99,820**	**64,698**	80,100	**18,372,236**
Crystal Springs @ SE 28th Ave	194	816	4.51*	4.51	**247,755**	**1,042,105**		
Crystal Springs @ mouth	129	345	12.80	13.00	**467,930**	**1,270,011**	37,900	**137,370,557**
Spring Ck @ Harrison Rd	236	261	2.50	2.12	**166,998**	**156,683**	64,800	**45,873,248**

In both 2012 and 2013, the spring-fed tributaries (Errol Springs, Crystal Springs, and Spring Creeks) located at the downstream end of Johnson Creek contributed the greatest *E. coli* and Bacteroides loads to Johnson Creek. Crystal Springs Creek at its mouth contributed 52% of the total calculated tributary loads of *E. coli* in 2012, 74% in 2013, and 63% of the Bacteroides load in 2012.

Upstream, notable *E. coli* contributions (>1%) were made by Badger, Sunshine, Thom, Thompson, Kelley, and Frog Creeks. The larger contributors were Kelley Creek, which contributed 5% of the total *E. coli* tributary loads in 2012 and 4% in 2013, and Sunshine Creek, which contributed 5% in 2012. Both Thom and Badger contributed 2% of the total E. coli loads in 2012 and 2013. Thom especially, as well as Thompson, and Frog Creeks are most surprising because of their very small size and summer streamflows. In 2012, Badger and Sunshine Creeks contributed the largest loads after the spring-fed tributaries, with 3% by Badger and 2% from Sunshine. Even though Butler Creek had very high *E. coli* and Bacteroides levels measured at sampling locations upstream of the mouth, streamflow measurements were not taken or estimated, so it does not show up as a high contributor in terms of load.

Bacteria source identification

Source identification for human and avian specific indicators yielded few sites from the 48 samples analyzed (Table 4). Only eight sites had quantifiable detections of human markers, and six samples had trace (below quantitative thresholds) concentrations indicating human waste (Figure 7). The avian marker was found in quantifiable concentrations at only five sites, and eight sites had trace concentrations. Despite the fact that six sites had verified avian fecal sources, many sites with waterfowl present did not yield quantitative results. This anomaly is discussed further under study limitations below.

Table 4. Quantifiable and trace detections of human and bird specific indicators in Johnson Creek mainstem and tributaries.

	Mainstem	Tributaries
Human specific indicator	2 detections, 1 trace	6 detections, 5 trace
Avian specific indicator	No detections	5 detections, 8 trace

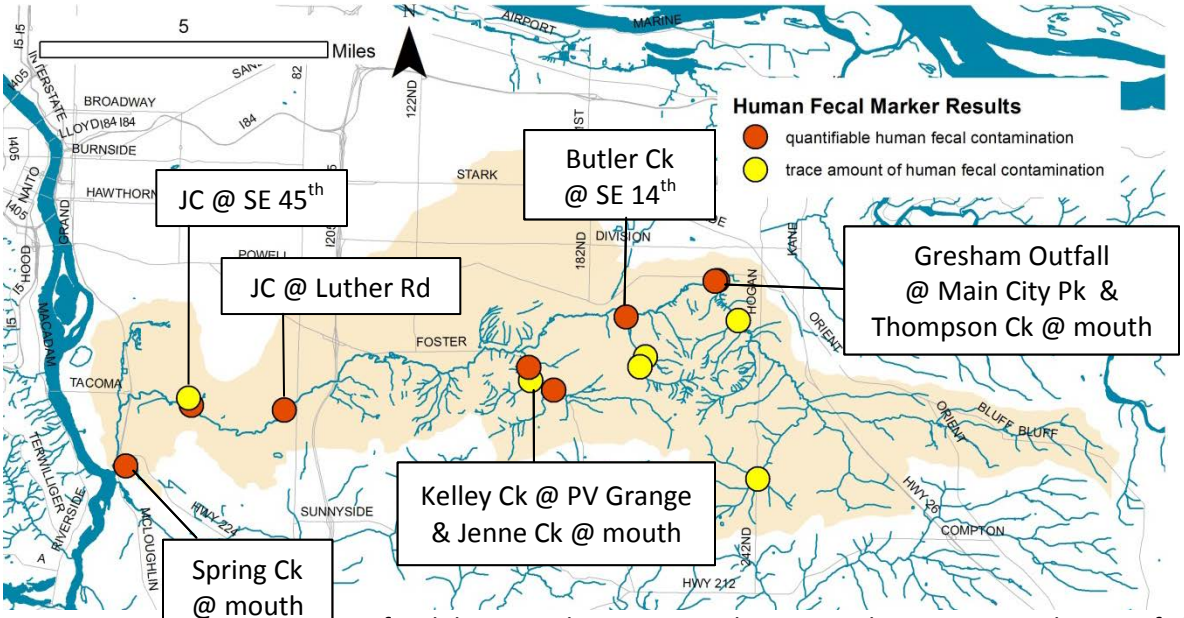

Figure 7. Map of human source fecal detection locations in Johnson Creek. Locations with quantifiable levels are called out.

There were only two detections of the Human Bacteroides ID (*B. dorei*) on the mainstem Johnson Creek. The highest level was at SE 45th Ave, which is located at the downstream end of a sizable area of cesspools and septic systems[2] . The other detection was at Luther Road, where a homeless camp is located.

[2] Clackamas County Water Environment Services recently sewered this area and properties are in the process of connecting to the public system.

On tributaries, the highest ratio of human marker to General Bacteroides was found in Butler Creek (see Table 6). Other creeks also had quantifiable levels of human source indicator bacteria, including Jenne Creek in Portland and Thompson Creek in Gresham. Additional tributaries with trace detections of human source indicator bacteria, just above the limit of quantification, were Spring Creek in Milwaukie, Kelley Creek at the Pleasant Valley Grange, and the Gresham Outfall at Main City Park.

Table 5 uses the same color coding as Figure 7 to show the three human markers analyzed – *Bacteroides dorei*, *B. stercoris*, and *B. thetaiotaomicron*. The most common, sensitive, and best tested marker is *B. dorei* on the top row. Samples that tested positive for *B. dorei* were further tested with the two less sensitive human markers in order to add evidence for human contamination.

Table 5. Results of three different Bacteroides species indicative of human fecal contamination.

	JC @ Regner	JC @ Luther	JC @ 45th	Butler @ 14th	Butler @ 27th	Butler @ Willow	Clatsop @ mouth	Errol Springs @ mouth	Jenne @ mouth	Kelley @ PV Grange	Spring Ck @ Harrison	Sunshine @ SV Rd	Thompson @ mouth	Outfall @ Main City Park
Bacteroides Dorei	**Trace**	218	3350	962	**Trace**	**Trace**	**Trace**	**Trace**	585	164	136	**Trace**	1490	199
B. stercoris	Absent	Absent	Absent	**Trace**	Absent	Absent	22	Absent	Absent	Absent	**Trace**	Absent	**Trace**	Absent
B. thetaiotaomicron	Absent	Absent	Absent	Absent	35	21	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

Human source bacteroides was found in several places where we know people recreate in the creek. The Spring Creek site, close to the mouth of Johnson Creek (Figure 6) is on Portland Waldorf School's campus in Milwaukie, and the Johnson Creek at SE 45th site is just upstream of Tideman Johnson Park. Thompson Creek and a storm outfall both enter Johnson Creek in Gresham's Main City Park, although the sample collected from mainstem Johnson Creek within Main City Park had low levels of *E. coli* and no human marker Bacteroides.

Figure 8 plots *E. coli* versus General Bacteroides, with the red dots indicating sites where a human source indicator marker was present above the limit of quantitation. There is little apparent relationship between *E. coli*, General Bacteroides, and Bacteroides species used as human markers, although the sites with the highest levels of *E. coli* or Bacteroides also contained human source indicator bacteria above the limit of quantitation. However, many human source hits also occurred at sites with lower levels of *E.coli* and General Bacteroides (note the red dots in Figure 8 within the lower green circle where most samples are near or below the 406 *E. coli* standard). This is similar to what was found by Sauer et al. (2011) in metropolitan Milwaukie, Wisconsin, where *E. coli* levels in streams and stormwater did not correlate with Human-host Bacteroides levels. In fact, a number of their samples with lower *E. coli* levels had high levels of Human host Bacteroides detections, indicating that Bacteroides may be a better indicator of human pollution.

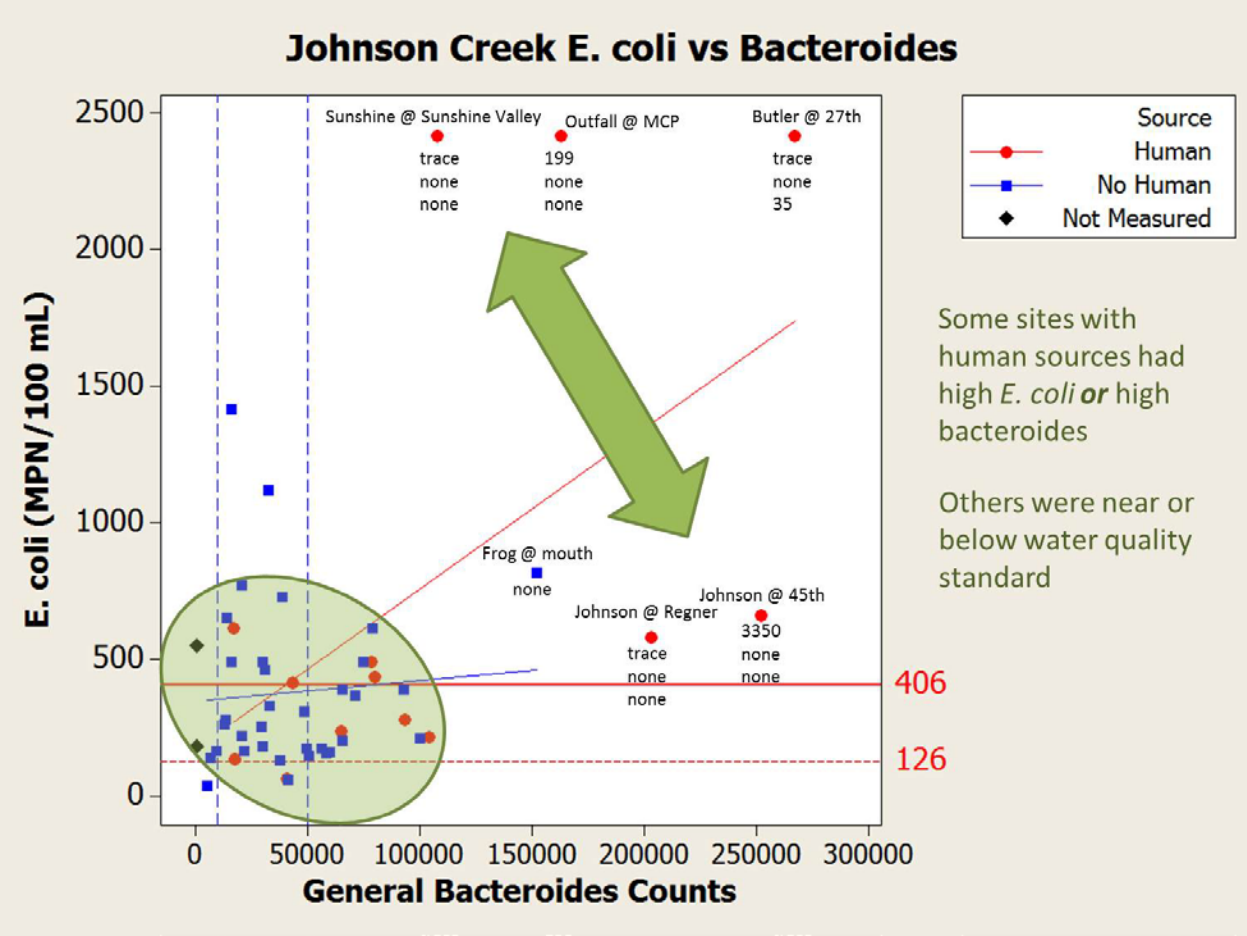

Figure 8. Scatterplot of *E. coli* versus General Bacteroides in the Johnson Creek watershed. Locations with high *E. coli* or General Bacteroides are labeled with the site name and the values for the three human source indicator bacteria evaluated - *Bacteroides dorei, B. stercoris*, and *B. thetaiotaomicron*

These qPCR-based human fecal source analyses provide qualitative evidence of human fecal contamination, but known ratios of General Bacteroides to Human-Sourced Bacteroides can be helpful in determining the likely sources of human fecal contamination. For example, Sauer et al. (2011) found that raw sewage has a human-marker Bacteroides to General Bacteroides ratio of about 5.1% in Milwaukee, Wisconsin. However, the ratio varies between populations. Source Molecular Corporation uses a baseline ratio of 1-3%, based on their own internal validation studies (Mauricio Larenas, pers. comm).

Based on this relationship, the ratio of bacteria found in the Butler Creek site (see Table 6) indicates that humans are likely the primary source of Bacteroides bacteria at that site. However, to pinpoint a more correct ratio for our area, analysis of raw sewage samples from the surrounding wastewater facilities and/or septic systems for General Bacteroides and Human Bacteroides ID would help gain a better understanding of the percentage of the human marker present within the local population. A more precise interpretation would be available with the submittal of such baseline samples (Mauricio Larenas, personal communication). A follow-up field investigation of human indicator bacteria in Butler Creek did not reveal any illicit connections within the lower Butler drainage, but potential contributions from homeless and septic systems in the upper Butler drainage are under investigation by the City of Gresham.

Lastly, a study by Soller et al. (2010) indicated that sites with more than 860 cells/100mL of Human Host Bacteroides may pose a detectable human health risk. Those sites with levels above this threshold are highlighted in Table 6.

Table 6. *E. Coli* and General Bacteroides for sites with quantifiable detections of *Bacteroides dorei*. Highlighted cells indicate moderate levels of bacteria that may be from human sources.

Sampling Location	*E. coli* (MPN/100 mL)	General Bacteroides ID (cells/100mL water)	Human Host Bacteroides ID (cells/100mL water)	Ratio, Human Bacteroides ID/General Bacteroides ID
Johnson Creek @ SE Luther Rd	488	78,600	218	0.28%
Johnson Creek @ SE 45th Ave	659	252,000	3350	1.33%
Butler @ SW 14th	132	17,800	962	5.41%
Jenne Creek @ mouth	58	40,900	585	1.43%
Kelley Creek @ Pleasant Valley Grange	613	16,900	164	0.97%
Spring Creek @ Harrison Rd	253	64,800	136	0.21%
Thompson Creek @ mouth	276	93,200	1490	1.60%
Gresham Outfall @ Main City Park	2419	163,000	199	0.12%

Study Limitations

The IDEXX *E.coli* method has an upper limit of 2420 org/100ml (without using dilution), thus the upper range of *E.coli* concentrations from samples that exceeded this level is unknown. Several samples from tributaries had the maximum *E.coli* concentration. Further sampling using dilutions to quantify the upper range of *E.coli* concentration is needed to determine whether significant *E.coli* spikes (and associated risks) occur in the watershed.

Where the range of *E.coli* concentration is within a range of 0 to 1000 org/100ml, it is difficult to assess whether there is a particular source of fecal bacteria. The presence of birds, rodents, and other wildlife in particular, beaver can contribute to background levels of *E.coli* above the water quality criteria. Many environmental factors can also contribute to elevated bacteria concentrations, including tributaries, residence time, temperature, and resuspension. These factors combined make source identification difficult in the range found in this study.

The Bird Fecal ID test, which uses a bird-specific indicator bacteria (Helicobacter), proved inconclusive. Only a few sites had positive results, and we found these results questionable as sites where large numbers of birds were observed in or near water had no detections of avian source bacteria. We proceeded to gather duck and goose fecal samples and mailed them to Source Molecular for analysis to

test their Bird Fecal ID (Figure 9). Despite the addition of locally-collected avian fecal material, the Bird Fecal ID marker test was still inconclusive. More data is needed to verify avian sources of bacteria in Johnson Creek.

Figure 9: Collecting avian fecal samples at Westmoreland Park.

The most reliable marker of human sourced fecal contamination is *Bacteroides dorei*, which has up to 94% human specificity (http://www.ncbi.nlm.nih.gov/pubmed/22809116; and http://www.sciencedirect.com/science/article/pii/S0043135413005502). While there is still a 6% chance that the source of *B. dorei* may not be human, utilizing multiple markers that are often associated with human sources can provide additional lines of evidence that detectable levels of these indicators could be associated with human sources. In samples where *Bacteroides dorei* was detected above the limit of quantitation, Source Molecular analyzed samples for *B. stecoris*, and *B. theraiotaomicron* as secondary validation. However, the results of the three Bacteroides species did not necessarily provide validation for human fecal sources because the secondary markers were generally absent from samples when the primary indicator, *B.dorei*, was present in quantifiable concentrations. In three samples, the secondary indicators were quantifiable where the primary indicator was found only in trace concentrations.

There was little evidence of human bacteria sources from areas in the upper watershed with known septic systems identified in this study, whereas SE 45th site showed higher levels of human fecal contamination, probably associated with nearby, leaky cesspools. This may be due to the mid-summer (low streamflow during low seasonal groundwater) sampling design where the unsaturated soil conditions may allow infiltration of leaking septage. We may expect to find septic impacts during wetter periods during the year.

Additional geographic analysis of known unsewered areas with our data could provide insights into areas with human fecal contamination detections. For example, using this technique, Peed et al. (2011) found a correlation between septic system density and levels of the Human Bacteroides ID marker in small streams in southwestern Ohio, but no correlation between septic system locations and *E. coli* levels.

Finally, one key drawback of the qPCR MST method is the lack the precision required for repeatable load calculations of fecal contamination. However, MST results can be effectively used to qualitatively identify those sources that are likely contributing more bacteria or are more abundant in the watershed and can therefore be prioritized for management or additional characterization. (Tetra Tech and Herrera, 2011).

Conclusions

Lessons Learned

- *Human fecal contamination is likely present in Johnson Creek.*
- *Some tributaries are fecal bacteria sources.*
- *The General Bird Fecal ID qPCR marker does not appear to be sensitive to local birds gut flora.*

Quantifying bacteria concentrations and identifying bacteria sources is challenging despite new scientific methods. Despite the number of samples and results, much of the data did not point to a clear conclusion. In the end, it was only possible to rank the detections as high, medium, and low levels of human fecal contamination, rather than quantifiable proportions of the total bacterial load.

One intriguing lesson learned was that the human bacterial source tracking marker can have high values even when *E. coli* is quite low. As a facultative anaerobic bacteria, *E. coli* may not be the best proxy for fresh fecal contamination, as it has the ability to survive outside a warm-blooded host.

In terms of mainstem Johnson Creek sites, we expect the human contamination at SE 45th Ave to decrease as nearby houses currently on cesspools are hooked up to the new sewer system. Furthermore, the site at SE Luther Road will possibly be addressed through restoration and park development starting in 2014 on the upstream property, which is currently a large homeless encampment. Follow up management actions and outreach are needed, though the results have been presented at UERC and published in the Johnson Creek Watershed Council newsletter (Fall 2012). This includes follow up reach-scale outfall and instream monitoring during additional wet and dry periods where human contamination was found, follow up stormwater samples from ditches in targeted watersheds, and watershed wide mapping of unsewered areas which might be used to develop a risk map of homes with older septic system built close to a stream.

In the end, much of the *E. coli* and General Bacteroides bacteria may be coming from beaver and other wildlife, rather than human sources. As of March, 2014, a new qPCR indicator for determining fecal contamination from beaver has become available, which could shed light on consistently high summertime *E. coli* and other bacteria levels in Johnson Creek. Recent funding by ODEQ to Clackamas Community College to develop a qPCR bacterial source tracking laboratory may provide less expensive and more locally-derived indicators for birds and perhaps other sources of fecal contamination.

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